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PERSPECTIVES IN BASIC SCIENCE

# Macrophages and progressive tubulointerstitial disease

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**Macrophages and progressive tubulointerstitial disease.** In chronic renal disease, tubulointerstitial inflammation and injury is associated with infiltrating macrophages. As a consequence of primary injury, proteinuria, chronic hypoxia, and glomerular-derived cytokines may all differentially modulate the expression of factors that promote macrophage recruitment. In addition to adhesion molecules and chemokines, products of complement system and renin-angotensin system activation may direct this process. Once present at interstitial sites, macrophages interact with resident cells and extracellular matrix to generate a proinflammatory microenvironment that amplifies tissues injury and promotes scarring. There is now increasing evidence for the efficacy of interventions directed against factors that recruit, activate, or are produced by macrophages. A detailed understanding of the biology of this area may lead to the further development of therapies that will improve the outcome of renal disease.

Over 30 years ago, Risdon et al first described the association between the degree of renal impairment and the extent of tubulointerstitial damage in patients with glomerular disease [1]. Subsequent studies have shown that this relationship is a major determinant of progression to end-stage renal failure (ESRF), and that interstitial inflammation has a central role in this process. As the interstitial macrophage is involved in both the initiation and continuation of this inflammatory response, a detailed knowledge of the role of these cells in situ may lead to the development of new treatments. Here we review our current understanding of the mechanisms that recruit monocytes to the interstitium, how interactions between the differentiated macrophage and other components of the local microenvironment promote progressive injury, and how current and future therapies may modulate these processes. For the purpose of this review, both monocytes and differentiated interstitial macrophages will be denoted by Mϕ.

**Key words:** macrophage, tubulointerstitial disease, progressive renal disease, trafficking, activation.

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## PRIMARY INJURY AND SECONDARY DISEASE

Tubulointerstitial (TI) disease is common to all chronic progressive renal diseases, irrespective of the initial trigger or site of injury. It is characterized by inflammatory cell infiltrates, loss of peritubular capillaries, atrophy of tubules, and interstitial scarring. The extent of disease on renal biopsy inversely correlates with renal function and accurately predicts renal prognosis [2]. Morphometric studies indicate that glomerular and tubulointerstitial injury is interdependent: thus, in glomerulonephritis, damage of a single nephron may progress from initial glomerular injury to peritubular and interstitial inflammation, tubular atrophy, and interstitial scarring [2]. This local inflammatory response may then involve adjacent tubules and so initiate or potentiate upstream glomerular injury. As damage progresses, remaining glomeruli compensate through the development of capillary hypertension, and glomerulosclerosis develops independent of the primary injury (reviewed in [3]). Thus, nephron loss as a consequence of secondary interstitial inflammation may progress to ESRF. A prominent feature throughout this process is the presence of leukocytes at sites of injury.

## LEUKOCYTES AND INTERSTITIAL INFLAMMATION

In the normal kidney there are small numbers of interstitial leukocytes. These are predominantly Mϕ, although T cells are also present. In human glomerular disease there are increased numbers of Mϕ and T cells at interstitial sites; most studies report that T cells predominate, and the majority of these are CD4+, although there is considerable variation between these analyses (Table 1). These results should be interpreted with caution, however, as Mϕ enumeration in these studies was performed by immunohistochemistry (IHC) with primary antibodies directed against the surface antigen CD14. As the expression of this antigen varies with the maturity and activation status of the cell, Mϕ numbers have probably been underestimated.

Only a small fraction of leukocytes in the normal kidney comprise B cells, natural killer (NK) cells, and neutrophils. In disease states they represent less than 10% of

**Table 1.** Interstitial Mϕ and T-cell ratios in renal disease: Published series with 10+ patients

| Nephropathy (Patient number) | Reference | T cell: Mϕ | CD4: CD8 |
|---|---|---|---|
| Lupus (35) | [4] | 1.5 | 1.5 |
| IgA (34) | [5] | 0.9 | 2.1 |
| Non-cresc IgA (18) | [6] | 2.4 | 1.4 |
| Cresc IgA (5) | | 1.1 | 1.3 |
| Memb (13) | [7] | 2.8 | 0.5 |
| FGS (13) | | 1.4 | 1.9 |
| DN (9) | | 4 | 1 |
| IgA (18) | | 2.4 | 1.4 |
| Lupus (13) | | 2.7 | 1.9 |
| Cresc GN (14) | | 2.9 | 1 |
| MPGN (8) | | 2.6 | 1.4 |
| FPGN (10) | | 3 | 1.2 |
| Memb (36) | [8] | 0.8 | 2.2 |
| MPGN (17) | [9] | 5.1 | – |
| Cresc GN (9) | [10] | 16 | 2 |
| FGS (11) | | 9.1 | 1.4 |
| MPGN (7) | | 3.8 | 1.6 |
| Cresc GN (3) | [11] | 1.1 | 1.5 |
| Lupus (7) | | 0.8 | 1.3 |

Abbreviations are: Cresc, crescentic; Memb, membranous nephropathy; FGS, focal glomerulosclerosis; DN, diabetic nephropathy; MPGN, membranoproliferative glomerulonephritis; FPGN, focal proliferative glomerulonephritis. Ratios extrapolated from published data where cell numbers had been expressed as number per $mm^2$ tissue viewed.

total infiltrating cells, although in proliferative glomerulonephritides, this proportion may be higher [7, 10].

Studies in animal models indicate that Mϕ are the dominant infiltrating cell in the initiation and progression of injury in chronic renal disease (reviewed in [12]). Strategies that limit disease progression in his setting include: (*1*) the systemic depletion of Mϕ; (*2*) inhibition of proinflammatory cytokines that both activate and are produced by activated Mϕ; and (*3*) the blocking of factors that promote the recruitment of Mϕ to tissue sites. While T cells almost certainly have an important role in progressive injury in situ in chronic TI disease, this has not been clearly defined to date, and factors that promote their recruitment and activation require further investigation. The use of T-cell–deficient mice in a number of models of progressive renal disease did not, however, prevent the development of renal injury [13–15].

## MACROPHAGE-INDUCED TUBULOINTERSTITIAL INJURY

Resident and infiltrating Mϕ play a central role in innate immune protection both through the clearance of infective pathogens and through the repair of tissue injury that occurs, in part, as a consequence of this response. For example, the initial response of Mϕ to bacterial infection is cytotoxic and proinflammatory; then, on control of the infection, Mϕ phagocytoze cellular debris and apoptotic bodies and begin tissue repair. However, in many noninfective renal diseases associated with Mϕ infiltrates,

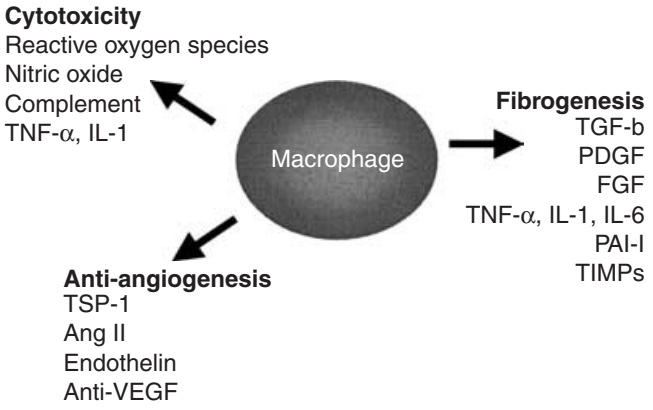


**Fig. 1. Secretory products of activated interstitial macrophages that may promote tubular atrophy, scar formation, and peritubular capillary loss in secondary tubulointerstitial disease.**

although the primary cause may abate, interstitial inflammation and TI injury worsens [12].

Direct damage to resident cells is caused through the generation by Mϕ of radical oxygen species (ROS), nitric oxide (NO), complement factors, and proinflammatory cytokines ([12]; see Fig. 1). Mϕ can also affect supporting matrix and vasculature through the expression of metalloproteinases and vasoactive peptides. Resident interstitial fibroblasts and myofibroblasts proliferate in response to Mϕ-derived profibrogenic cytokines, and their number correlates with the subsequent formation of a scar and renal decline [16]. These cells are a primary source of the extracellular matrix (ECM) proteins that accumulate to form a scar. They may be derived from transdifferentiated tubular epithelial cells, a process promoted by profibrogenic cytokines, including transforming growth factor-β (TGF-β) expressed by Mϕ, and also by tubular epithelial cells as a consequence of Mϕ-tubular cell interactions (reviewed in [17]). The role of TGF-β has been studied in detail. It promotes the production of all the major matrix proteins by fibroblasts, inhibits expression of matrix degrading plasminogen-activator inhibitor (PAI), and increases the activity of tissue inhibitors of metalloproteinases (TIMPS). In a rat model of progressive proteinuric renal disease, interstitial Mϕ produced TGF-β, and levels correlated with interstitial inflammatory infiltration [18].

## MONOCYTE RECRUITMENT TO TISSUE SITES

Although interstitial Mϕ proliferate in situ, their increase in numbers at sites of secondary TI disease primarily reflects the recruitment of circulating Mϕ, which largely occurs at the level of postcapillary venules (Fig. 2). Endothelial cells (EC) and the subendothelial environment at sites of disease variably demonstrate constitutive, up-regulated, and de novo expression of a

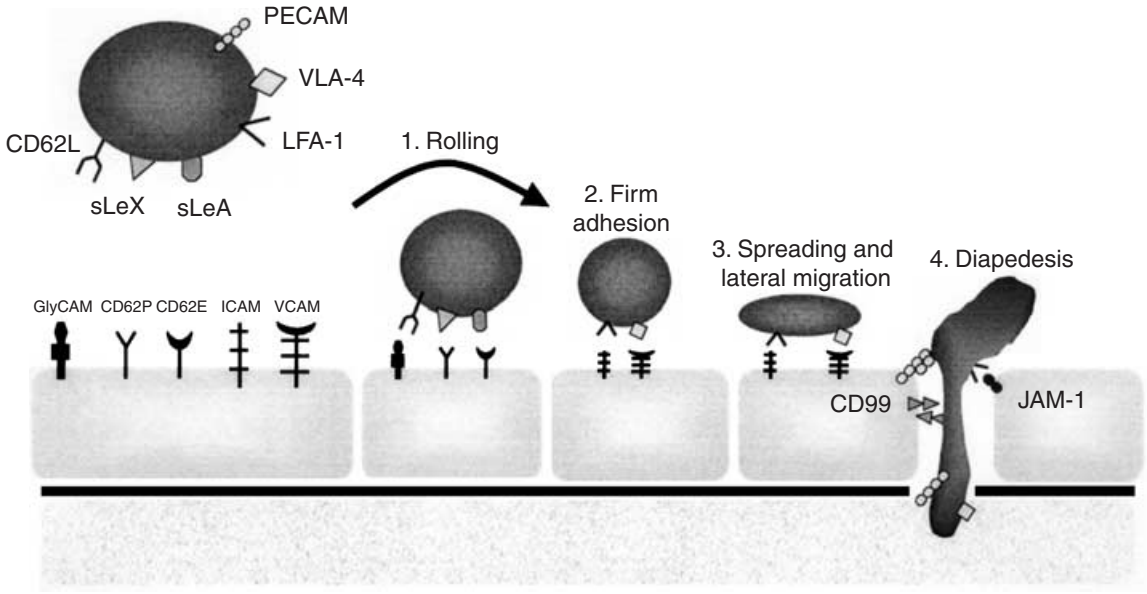


**Fig. 2. An overview of monocyte trafficking: (*1*) selectin-mediated rolling of the leukocyte rolling on the EC surface; (*2*) integrin-mediated firm adhesion to EC surface; (*3*) integrin-mediated spreading and migration to the EC junction; (*4*) integrin, PECAM-1, CD99, and JAM-1–mediated diapedesis.** Chemokines immobilized at the EC or in solution have a biological role at each step.

number of ligands that direct this process. The binding of these molecules to counter-receptors on the surface of Mϕ promotes the phenotypic and ultrastructural changes required for cell transmigration. There are several obligate steps in this process, each of which is directed by specific families of molecules (reviewed in [19]).

### Selectin-mediated rolling

Following their initial contact with the luminal surface of venules, the cells roll through the rapid association and dissociation of EC selectins and Mϕ counter ligands (Fig. 2, Table 2). These interactions are strong enough to resist shear stress, thereby slowing the cells sufficiently to permit sampling of the rich microenvironment at the EC surface (reviewed in [20]).

Three selectins have been identified. P-selectin is stored preformed in Weibel-Palade bodies, and is rapidly translocated to EC surface on activation (by proinflammatory cytokines or histamine); this expression is transient and decreases in minutes. E-selectin expression is largely restricted to EC that have been activated by proinflammatory cytokines such as IL-1β and TNF-α; expression is delayed until de novo mRNA and protein synthesis is complete (4–6 hours). L-selectin is constitutively expressed by most leukocytes, as well as EC. It has a central role in normal T-cell recirculation, and is also involved in Mϕ recruitment at sites of inflammation [20].

Selectins bind carbohydrate counterligands through an N-terminal C-type lectin domain. The ligands for E- and P-selectin, which are constitutively expressed on Mϕ, are closely related to sialyl Lewis X (sLeX) and sialyl Lewis A (sLeA). L-selectin can also bind these ligands and sulphated polysaccharides such as GlyCAM-1, MadCAM-1, and heparan sulfate proteoglycans (HSPGs) on EC and ECM [19].

### Integrin-mediated adhesion

Integrins are heterodimeric receptors that are constitutively expressed on Mϕ. They are activated by conformational changes triggered following ligation of receptors on rolling Mϕ by molecules (including chemokines) sequestered at the EC surface (see below). The cell then firmly adheres to the EC through interactions between the β1 integrin, very late activation antigen-4 (VLA-4), and the β2 integrin, leukocyte functional antigen-1 (LFA-1), and their respective counter-receptors vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). These are constitutively expressed by ECs at low levels, and heavily up-regulated by proinflammatory cytokines such as IL-1β, interferon-γ (IFN-γ), and TNF-α [19].

### Transendothelial migration

Following firm adherence to EC, Mϕ elongate through extension of lamellipodium to form leading edge adhesive complexes that provide traction. The cells then crawl along gradients of chemotactic factors and adhesion molecules to intercellular junctions, where there is diapedesis to the subendothelial space. VLA-4 preferentially mediates lateral migration along the endothelium to the intercellular junction; LFA-1 primarily mediates diapedesis [21].

**Table 2.** Adhesion molecules involved in monocyte trafficking

| Stage of trafficking | Family | Individual members | CD number | Ligands |
|---|---|---|---|---|
| Rolling | Selectins | P-Selectin | CD62P | PSGL-1, L-selectin |
| | | E-Selectin | CD62E | ESL-1, PSGL-1,L-selectin |
| | | L-Selectin | CD62L | sLex, GlyCAM-1 |
| Firm adhesion | Integrins | LFA-1 | CD11a/Cd18 | ICAM-1 |
| | | VLA-4 | CD49d/CD29 | VCAM-1 |
| Transendothelial migration | Integrins | LFA-1 | CD11a/Cd18 | ICAM-1 |
| | | VLA-4 | CD49d/CD29 | VCAM-1 |
| | Ig-SF | PECAM-1 | CD31 | PECAM-1 |
| | | JAM-1 | | LFA-1 |
| | Glyco- protein | | CD99 | CD99 |
| Subendothelial migration | Integrins | VLA-4 | CD49d/CD29 | FN (CS-1) |
| | | VLA-5 | CD49e/CD29 | FN (RGD) |
| | Ig-SF | PECAM-1 | CD31 | Basement membrane components |

Abbreviations are: FN, fibronectin; Ig-SF, immunoglobulin superfamily.

The first stages of diapedesis are directed by homophilic interactions of platelet-endothelial-cell adhesion molecule-1 (PECAM-1), expressed on the luminal side of EC and intercellular junctions. Deeper penetration of Mϕ through the clefts between EC is then promoted by CD99. Both PECAM-1 and CD99 are constitutively expressed; this is in contrast with junctional adhesion molecule-1 (JAM-1), a ligand for LFA-1. In quiescent EC, JAM-1 is exclusively localized to tight junctional complexes and involved in permeability control. On cell activation, JAM-1 is relocated to the apical aspect of ECs with a resultant alteration in tight junction structure and increased permeability to leukocytes (reviewed in [22]).

Subsequent passage through the basal lamina is dependent on contact between ECM and Mϕ expressed PECAM-1 [23]. Further interactions between VLA-4 and VLA-5 and underlying matrix then facilitate tissue migration along a bioactive gradient [24]. While the processes at this stage are less well characterized than those that promote transendothelial migration, chemokines sequestered on ECM at an increasing concentration toward inflammatory areas have a central role.

## Chemokines

Chemokines are small chemotactic cytokines that direct leukocyte recruitment in inflammation and homeostasis through ligation of chemokine receptors expressed on the surface of leukocytes. To date, over 40 chemokines and 19 chemokine receptors have been identified. Chemokines are classified by a nomenclature (CX3CL, CXCL, CCL, and CL) that describes the relative positions of the first 2 conserved cysteine (C) residues of the molecule to any other residue (X) in the 4 cysteine motif common to all chemokines. This is reviewed in detail elsewhere [25]. For chemokine receptors, the nomenclature used reflects the class of ligating chemokines: that is, CX3CR, CXCR, CCR, and CR. Many chemokines can bind to several different receptors within a subclass, and receptors may bind several different chemokines. However, differential chemokine expression at inflammatory sites and differential lineage and activation-dependent receptor expression by leukocyte subsets confers specificity in recruitment to tissue sites.

Stimuli for the expression of inducible chemokines are diverse and include proinflammatory cytokines (e.g., TNF-α, IL-1β, IFN-γ), immune complexes, complement activation products, and nonimmune stimuli such as shear stress. Chemokines presented to circulating Mϕ may be produced by EC or originate locally from other sources; for example, extravascular chemokines may be internalized at the abluminal surface of ECs and transcytozed for luminal presentation.

The rolling of Mϕ on EC selectins may allow exposure to inflammatory chemokines whose modes of presentation and differential receptor targeting may initiate disparate effects. For example, growth-related oncogene-α (GRO-α/CXCL1) is immobilized on the EC surface by HSPGs; it ligates CXCR2 to rapidly convert rolling to firm adhesion by activating Mϕ integrins. Conversely, monocyte chemoattractant protein-1 (MCP-1/CCL2) is expressed unbound in a soluble form; through CCR2, it mediates Mϕ shape change, spreading, and subsequent transendothelial migration [26]. Some chemokines may act both on adhesion and chemotaxis; for example, regulated upon activation, normal T cell expressed and secreted (RANTES/CCL5) is presented by EC proteoglycans, and acts through CCR1 to promote integrin-mediated firm adhesion. This chemokine can then support Mϕ spreading and shape change primarily through CCR5. Subsequent transendothelial migration in response to soluble RANTES/CCL5 is then directed by both CCR1 and CCR5 [27].

MCP-1/CCL2 and RANTES/CCL5 differentially and selectively regulate the avidity of Mϕ expressed integrins. They induce early activation and deactivation of VLA-4 to facilitate transendothelial diapedesis, and late and persistent VLA-5 activation to mediate subsequent interactions with matrix proteins in the basement membrane

**Table 3.** Monocyte directed chemokines in situ in human secondary tubulointerstitial disease

| Chemokine | CC nomenclature | Source | Reference | Targeting chemokine receptor |
|---|---|---|---|---|
| MCP-1 | CCL2 | TEC, MNC, PTC | [31–37] | CCR2 |
| MCP-4 | CCL13 | TEC, MNC, PTC | [38] | CCR2 |
| RANTES | CCL5 | MNC, TEC | [31, 34] | CCR1, CCR5 |
| MIP-1α | CCL3 | TEC | [31] | CCR1, CCR5 |
| MIP-1β | CCL4 | TEC | [31] | CCR1, CCR5 |
| IL-8 | CXCL8 | TEC | [39] | CXCR2 |

Abbreviations are: TEC, tubular epithelial cell; MNC, mononuclear cell; PTC, peritubular capillary; MIP, macrophage inflammatory protein.

and ECM [24]. Following migration to the subendothelial space, movement along a chemokine gradient may be facilitated by expression of matrix degradative enzymes by Mϕ. Both MCP-1/CCL2 and RANTES/CCL5 are capable of inducing this production, further amplifying the potential for recruitment of subsequent waves of leukocytes to inflammatory sites [28].

## Mϕ-DIRECTED ADHESION MOLECULES AND CHEMOKINES IN SITU

### Human tubulointerstitial disease

*Selectins.* P-selectin and E-selectin are present on peritubular and glomerular capillaries in patients with glomerulonephritis of various causes, but not detectable in the tubulointerstitium or glomeruli of renal tissue obtained from normal human kidneys [20]. The level of expression in disease correlates with the extent of the interstitial inflammatory infiltrate and degree of tubular atrophy and scar formation. L-selectin expression by interstitial cells has been demonstrated in disease, and correlates with the infiltration of Mϕ [20]. Ligands for L-selectin, including sLex, are found at the corticomedullary junction in human glomerulonephritides, and expression relates to interstitial leukocyte infiltration [29]. The corticomedullary junction is also a preferential site of P- and E-selectin expression [20].

*Integrins and their ligands.* There is low-level expression of ICAM-1 by peritubular capillaries in normal kidney. This expression is heavily increased in disease states, with increased vascular expression and de novo expression by tubular epithelial cells; this correlates with inflammatory cell infiltration and the extent of tubulointerstitial injury. Infiltrating interstitial cells also express ICAM-1 and its counterligand, LFA-1 [19].

VCAM-1 is expressed at low levels on peritubular capillaries and tubular epithelium in normal kidneys. In disease, this expression is increased at both sites and correlates with the extent of tubulointerstitial damage and VLA-4 expression on infiltrating leukocytes [19].

*Chemokines.* A number of chemokines have been identified in secondary TI disease (reviewed in [30]). The expression of chemokines active against Mϕ is listed in Table 3. The majority of studies have focused on MCP-1/CCL2 and RANTES/CCL5.

**MCP-1/CCL2.** This chemokine is expressed by tubular epithelial cells (TEC), infiltrating monocytes, and peritubular capillary endothelial cells in a number of renal diseases. Expression in situ, and in the urine of patients with chronic progressive renal disease, correlates with interstitial macrophage infiltration and fibrosis [31–37]. Urinary MCP-1/CCL2 is probably primarily derived from tubulointerstitial sources in these nonproliferative diseases, as glomerular tuft expression is not detected (personal observation; Fig. 3E and F). Interstitial cells have been identified by IHC and by in situ hybridization (ISH) to express the counter-receptor CCR2 (personal observation [40]). By dual-staining IHC, CCR2 expression in the interstitium of nonproliferative renal disease is restricted to Mϕ (personal observation, Fig. 3G and H).

**RANTES/CCL5.** There is increased TEC expression and an associated interstitial mononuclear cell inflammatory infiltrate in membranous nephropathy, while in proliferative glomerulonephritides, expression is predominantly confined to the glomerulus [31, 34]. The expression of CCR5 in the interstitium of patients with either proliferative or nonproliferative renal disease is restricted to T cells. In contrast, interstitial cells expressing CCR1, an alternative receptor for RANTES/CCL5, are predominantly Mϕ, and their numbers correlate with urinary levels of the chemokine [41, 42].

### Animal models and secondary tubulointerstitial disease

*Selectins.* In a rat model of obstructive nephropathy there was increased expression of P-selectin on peritubular capillaries and the vasa recta in the outer and inner medulla [43]. This was associated with Mϕ infiltration, which was significantly decreased by a blocking antibody to P-selectin. In this study, E-selectin and sLeX-related ligands for L-selectin were not expressed in the obstructed kidney, although others have demonstrated an increased expression of the L-selectin non-sLeX ligand versican in the interstitium and peritubular capillaries in this model [44].

*Integrins and their ligands.* In rodent chronic progressive proteinuric renal disease induced by puromycin aminonucleoside (PAN), ICAM-1 expression was increased in the interstitium, particularly on infiltrating mononuclear cells [45]. In the remnant kidney and

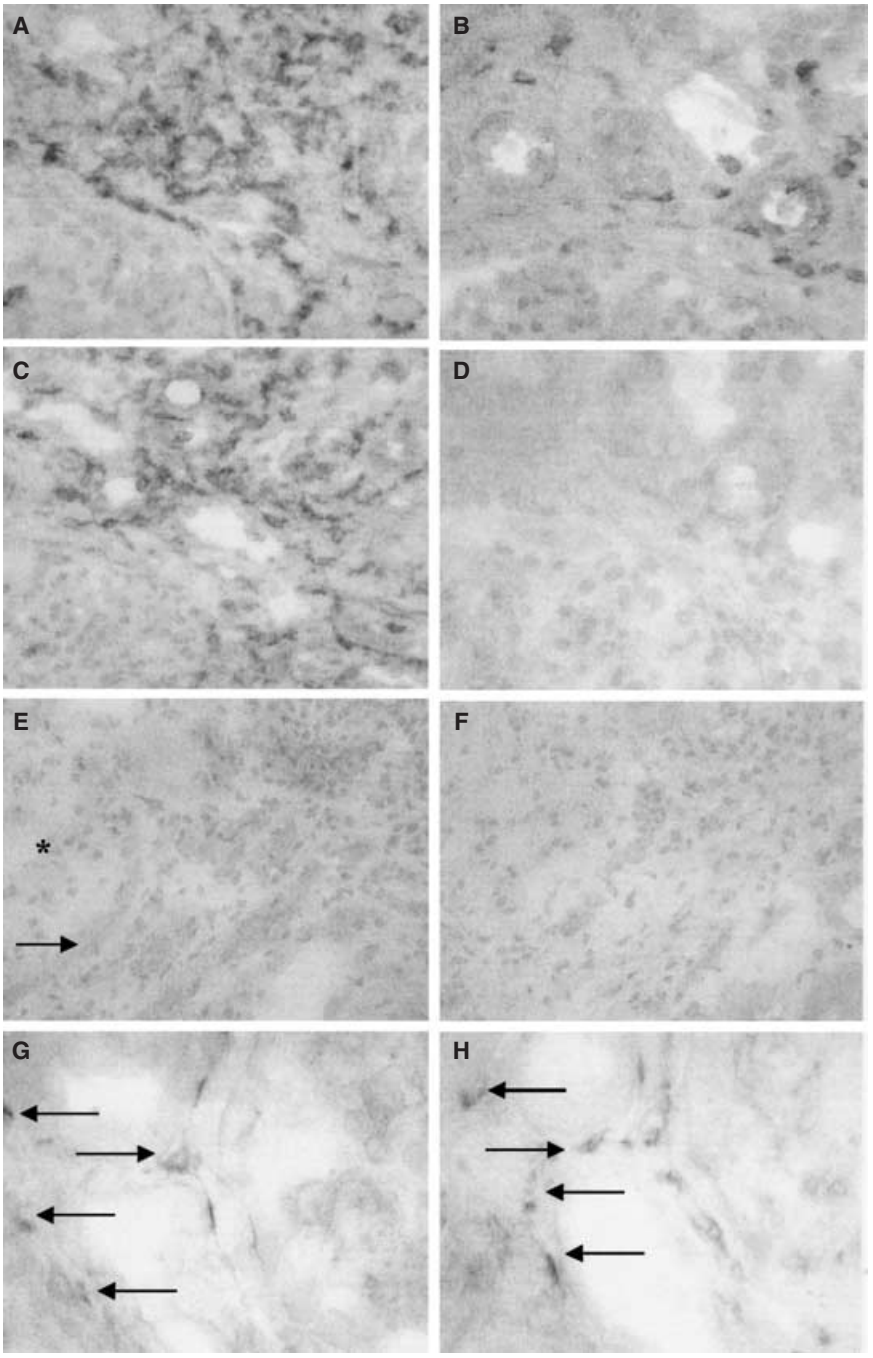


**Fig. 3. Dual staining IHC demonstrating (*A*) CD68+ve macrophages, (*B*) CD3+ve T cells, and (*C*) their colocalization, in the interstitium in advanced ischemic nephropathy (×400).** The methodology utilized the 3-step indirect IHC technique on serial sections, initially staining for anti-CD68 (5 μg/mL; clone PG-M1; Dako, Ltd., Ely, UK) and visualizing with DAB (brown pigment), followed by staining for anti-CD3 (30 μg/mL clone UCHT-1; Dako) and visualizing with Fast Blue (blue pigment) (C). The controls used were serial sections with the replacement of either the first (B), second (A), or both (*D*) antibodies with an isotype control at the identical concentration. (*E* and *F*) Immunohistochemistry demonstrating MCP-1/CCL2 expression in advanced IgA nephropathy (×400). MCP-1/CCL2 is expressed predominantly by tubular epithelial expression in chronic nonproliferative nephropathies. There is no glomerular tuft (∗) expression, and little glomerular epithelial expression (arrow) [anti-MCP-1; AF-279-NA; 10 μg/mL; R&D Systems, UK (E); isotype (F)]. (*G* and *H*) Dual staining IHC demonstrating CCR2 expression by interstitial macrophages in membranous nephropathy. CCR2 expression [anti-CCR2; clone 5A11; 20 μg/mL; gift from LeukoSite, Cambridge, MA; DAB (brown) visualization] (G and H) colocalizes with CD68 expression (Blue, Fast Blue visualization) to produce black color (arrows) (H). Methodologies as described for 3A-D. Studies performed by K. E.

obstructive models, adhesion molecule expression by peritubular capillaries, tubular epithelial cells, and infiltrating leukocytes was increased [46, 47]. In the obstructed kidney, antisense oligonucleotides predominantly blocked tubular epithelial cell expression of ICAM-1. This was associated with significantly reduced Mϕ infiltration and scar formation [47].

Tubulointerstitial ICAM-1 expression was demonstrated in accelerated anti-GBM disease, with expression by peritubular capillaries, tubular epithelial cells, interstitial fibroblasts, and infiltrating inflammatory cells [48]. Mononuclear cells expressing LFA-1 were adherent to ECs expressing ICAM-1 and also present at interstitial sites. Studies on the effects of integrin/ligand blockade have primarily concentrated on glomerular infiltration and injury.

*Chemokines.*

**MCP-1/CCL2.** Expression patterns are similar to those found in human disease. An overview of studies is provided in Table 4; these consistently indicate a role in Mϕ recruitment and tubulointerstitial disease progression. Other models have provided indirect evidence by

**Table 4.** Animal models of renal disease and tubulointerstitial MCP-1/CCL2, and beneficial therapeutic studies

| Animal model | Reference | Intervention |
|---|---|---|
| NTS GN | [49] | MCP-1 blocking Ab |
| Mouse | [50] | MCP-1 knockout |
| Rat | [51] | MCP-1 blocking Ab |
| | [52] | MCP-1 OGN antisense |
| (Lupus GN) | | |
| MRL-Fas (lpr) mouse | [53] | MCP-1 knockout |
| NZB/W F1 mouse | [54] | Cyclophosphamide |
| Immune complex GN rat | [55] | Quinapril (ACE I) |
| Puromycin aminonucleoside nephrosis (PAN) rat | [56] | MCP-1 blocking Ab |
| Protein overload proteinuria rat | [57] | |
| | [58] | Anti-MCP-1 gene therapy |
| Hypertension 2K1C model rat | [59] | Valsartan (ARB) |
| Obstruction | | |
| Rat | [60] | |
| Mouse | [61] | Anti-MCP-1 gene therapy |
| | [62] | CCR2 knockout and CCR2 blockers |
| | [63] | |
| | [64] | |
| Subtotal nephrectomy rat | [65] | |
| | [66] | Protein restriction |
| | [46] | Enalapril (ACE I) |
| | | Candesartan (ARB) |
| | [67] | Lisinopril (ACE I) |
| (Membranous nephropathy) Heymann nephritis rat | [81] | Lisinopril (ACE I) |

Abbreviations are: NTS, nephrotoxic serum nephritis; GN, glomerulonephritis; ACE I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; OGN, oligonucleotides.

demonstrating that renoprotective strategies are associated with decreased TI MCP-1/CCL2 expression and disease progression (see Table 4).

**RANTES/CCL5.** Increased expression is present in acute and chronic disease [30]. Studies in obstructive and proteinuric nephropathy indicate that infiltrating leukocytes are a significant source of the chemokine [61]. These investigators also found that interstitial T cells predominantly expressed CCR5, while interstitial macrophages expressed CCR1, consistent with the findings in human renal disease [41, 42]. The use of CCR1 knockout animals or CCR1 blockade was associated with a reduction in both inflammatory infiltrate and the development of fibrosis [68, 69]. Conversely, using CCR5 knockout animals did not affect the development of TI disease in the obstructive nephropathy model. In the murine nephrotoxic serum model of proliferative glomerulonephritis, blockade of RANTES/CCL5 decreased interstitial Mϕ infiltration but not scar formation, whereas blocking MCP-1/CCL2 decreased both [49].

## OTHER MECHANISMS OF MONOCYTE RECRUITMENT IN SECONDARY TI DISEASE

### Complement activation products

Activation of the complement system in situ leads to Mϕ recruitment by (*1*) release of C5a, which is directly chemotactic and causes Mϕ integrin activation, and (*2*) production of the membrane-attack-complex (C5b-9); this can activate resident renal cells to express proinflammatory cytokines and promote classic recruitment pathways (reviewed in [70]).

Both C3 and C5b-9 have been demonstrated in the glomeruli of renal tissue from patients with primary immune-mediated glomerular diseases, indicating activation of the classical pathway [70]. C5b-9 is also found at tubular sites in these diseases, which may indicate uptake of filtered complement components. However, the presence of tubular C5b-9 may also represent alternative pathway activation at this site, as despite no glomerular expression, tubular C3 and C5b-9 is present, and C5b-9 is detected in the urine in some non-immune–mediated renal diseases [70]. Notably, the proximal tubule has inherent brush border C3 convertase-like activity, and is relatively deficient in complement inhibitory proteins. Further, factor B, which triggers initial activation of the alternative pathway, is expressed by tubular epithelial cells in vitro in response to proinflammatory cytokines [71]. Thus, essential complement proteins (such as C3) present in glomerular filtrate in nonselective proteinuric renal disease may be activated at this site. Also, proximal tubular cells may generate C3 in response to cytokines and filtered proteins or C5b-9 [71]. In human renal disease there is a correlation between tubular C5b-9 deposition, interstitial Mϕ infiltration, tubular atrophy, and

**Table 5.** Animal models of renal disease, tubulointerstitial osteopontin, and beneficial therapeutic studies

| Animal model | Reference | Intervention |
|---|---|---|
| NTS GN - rat | [84]<br>[85] | OPN OGN antisense<br>OPN blocking Ab |
| (Lupus GN) - MRL-Fas (lpr) mouse | [86] | |
| Puromycin Aminonucleoside Nephrosis (PAN) rat | [87] | |
| Protein Overload Proteinuria rat | [57] | |
| Angiotensin II Infusion rat | [88] | |
| Obstruction<br>Mouse<br>Rat | <br>[89]<br>[90]<br>[91] | <br>OPN knockout<br>Angiotensinogen and AT(1) OGN antisense |
| Subtotal nephrectomy rat | [92] | |
| (Diabetic nephropathy)<br>Streptozotocin rat<br>db/db mouse | [93]<br>[80] | Perindopril (ACE I) |

Abbreviations are: NTS, nephrotoxic serum nephritis; GN, glomerulonephritis; OPN, osteopontin; ACE I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; OGN, oligonucleotides; AT(1), angiotensin 1.

interstitial fibrosis [70]. Increased levels of urinary C5b-9 levels in immune-mediated human renal diseases are associated with a worse clinical prognosis [72].

Animal models support these data. In murine immune complex–mediated glomerulonephritis, the absence of the C5a receptor was associated with significantly reduced interstitial mononuclear cell infiltrate and injury [73]. There was no difference in glomerular pathology. A previous study using a similar model had showed that absence of C5 attenuated both glomerular and TI disease [74]. These observations indicate a role for C5b-9 formation in glomerular injury and for C5a, through recruitment of mononuclear cells, in TI disease. In nonimmune renal disease, however, C5b-9 has a more important role in interstitial Mϕ recruitment. The use of C6 knockout animals in the PAN and remnant kidney models was associated with reduced interstitial inflammation and TI injury [75, 76]. The absence of C6 prevents the formation of C5b-9 but not other upstream components, such as C5a. This same group had previously demonstrated in the PAN model that inhibiting tubular expression of the complement regulatory protein Crry by antisense oligonucleotides augmented tubulointerstitial injury [77].

## Macrophage-colony stimulating factor-1 (M-CSF)

M-CSF promotes the differentiation and proliferation of bone marrow–derived monocytes to mature Mϕ, and is also a potent Mϕ chemoattractant. It also augments the proinflammatory and cytotoxic response of Mϕ to stimuli [78]. De novo expression of M-CSF protein in human renal disease has been demonstrated by IHC. Glomerular expression is restricted to mesangial cells in glomerulonephritides, with the highest levels in proliferative disease [79]. This expression correlates with local Mϕ infiltration, proliferation, and activation. In both glomerular and nonglomerular renal disease there is de novo expression of M-CSF by proximal and distal TECs, which also correlates with local Mϕ infiltration and proliferation [79].

The expression of M-CSF in animal models of immune and nonimmune renal disease is consistent with human disease [80, 81]. Direct evidence of a role for M-CSF in vivo has been recently published. Using the unilateral ureteric obstruction model of progressive renal disease, mice deficient of M-CSF or treated with an M-CSF receptor-blocking antibody had significantly reduced interstitial Mϕ infiltration and proliferation and tubular injury compared to control animals [78].

## Osteopontin

Osteopontin is a secreted phospho-glycoprotein with a diverse range of biological roles, including modulation of Mϕ function (reviewed in [82]). In vitro it is a chemoattractant and adhesion molecule for Mϕ. In vivo, a Mϕ infiltrate accumulates at the site of dermal injection of osteopontin in rodents. In humans, there is some constitutive expression by distal tubular cells in normal kidney. Increased expression at these sites and de novo expression by proximal TEC occurs in glomerulonephritis and correlates with the extent of Mϕ infiltration [83]. A number of animal models have been used to demonstrate the association between increased TEC osteopontin expression and interstitial inflammation, and there is direct evidence of a role in Mϕ recruitment in some studies (Table 5).

The effects of osteopontin on Mϕ are mediated through numerous receptors, including β1, β2, and β3 integrins, and isoforms of the hyaluronic acid receptor

CD44 [82]. Post-translational modification of osteopontin is common, and may be important in determining its biological effects. Cleavage of osteopontin by thrombin, which is likely to occur at sites of inflammation, reveals Mϕ binding sites, and also releases chemotactic fragments. Interactions between the C-terminal domain of osteopontin and isoforms of the receptor CD44 induces Mϕ chemotaxis, and interactions between the nonoverlapping N-terminal domain, revealed by thrombin cleavage, and beta(3)-integrins, leads to Mϕ spreading and activation [82].

### T cells

T cells expressing activation markers infiltrate the interstitium and colocalize with Mϕ in disease, but their role in the progression of tubulointerstitial injury is not clearly defined (personal observation [94], see Fig. 3A to D). Animal models using T-cell deplete animals developed the same amount of interstitial Mϕ accumulation and TI injury as T-cell replete controls [13–15]. More recent evidence has shown that activated T cells express proinflammatory cytokines that induce TEC expression of Mϕ-directed chemokines, such as MCP-1/CCL2 and RANTES/CCL5 [95]. This response is augmented in part by binding of TEC CD40 by T-cell CD40-ligand (CD40L). In an animal model of nonimmune progressive proteinuric renal disease, blocking CD40-CD40L interactions was associated with a significant reduction in both interstitial Mϕ recruitment and the development of tubulointerstitial injury [96].

CD4+ T cells predominate over CD8+ T cells at interstitial sites in the majority of progressive chronic renal diseases, although there is some variation (Table 1). Harris et al have shown that these subsets may have disparate roles. In the adriamycin nephropathy model of nonimmune progressive proteinuric renal disease, CD8+ T-cell depletion was associated with reduced Mϕ infiltration and tubulointerstitial injury, whereas the converse occurred when CD4+ T cells were depleted [97, 98].

### Inflammatory lipids

Kees-Folts et al demonstrated the presence of a lipid chemotactic for Mϕ in the urine of rats with protein-overload–induced proteinuria [99]. This lipid was derived from the tubular metabolism of albumin-borne fatty acids. Indeed, BSA depleted of bound fatty acids induced less interstitial inflammation and TI injury than lipid replete BSA [100]. In minimal change disease, urinary albumin is relatively depleted of fatty acid, and this has been proposed as an explanation for the absence of interstitial injury in this condition [101]. An inflammatory lipid has not been identified in human progressive proteinuric renal disease to date, however; observations in animal studies may relate to the heterogeneous albumin and not provide an explanation for the development of human disease.

Recently, urinary levels of liver-type fatty acid–binding protein (L-FABP), an intracellular carrier protein of free fatty acids (FFA) expressed in the proximal tubule of human kidney, have been shown to predict renal disease progression [102]. It is not clear if these findings reflect increased FFA tubular load, or if this is another marker of proteinuria-induced tubular injury.

### The renin-angiotensin-system

There are several direct proinflammatory effects of renin-angiotensin-system (RAS) activation. Angiotensin II (Ang II) is directly chemotactic for Mϕ, increases adhesion molecule expression on ECs and proximal TECs, and induces MCP-1 and osteopontin expression in situ [103, 104]. Mϕ chemotaxis and activation may also occur as a consequence of proximal TEC expression of TNFα, macrophage migration inhibitory factor (MIF), and reactive oxygen species in response to Ang II [104]. Ang II can also activate Mϕ directly to express proinflammatory cytokines and reactive oxygen species [105]. All of these effects are mediated through angiotensin II receptor type 1 (ATR1).

In the normal physiologic state, renal renin activity is restricted to the juxtaglomerular apparatus. It acts on circulating angiotensinogen to promote the systemic release of Ang II. In the subtotal nephrectomy model there was also de novo renin activity by TEC, which colocalized with increased Ang II production [106]. ACE inhibition reduced tubular renin activity, Ang II production, and tubulointerstitial injury, indicating that tubular RAS activity promotes disease progression. Indeed, proximal TECs can synthesize all the components of the RAS, resulting in high concentrations relative to plasma of Ang II in proximal tubular luminal fluid [107]. This has been observed in both human disease and animal models [91, 106, 108].

Macrophages can synthesize all RAS components [109]. Interstitial Mϕ in the subtotal nephrectomy model expressed Ang II and ATR1, indicating a potential feedback mechanism that promotes Mϕ recruitment and activation [110].

In chronic renal disease, hyperaldosteronism is often present as a consequence of adrenal secretion in response to changes in potassium homeostasis, induced by renal injury. Local production of aldosterone has not been described, in contrast to cardiac disease, where there is significant in situ generation (reviewed in [111]). The inflammatory effects of aldosterone include increased renal expression of proinflammatory cytokines, such as osteopontin and MCP-1/CCL2, with interstitial Mϕ infiltration and tubular injury [112]. Some of the beneficial effects

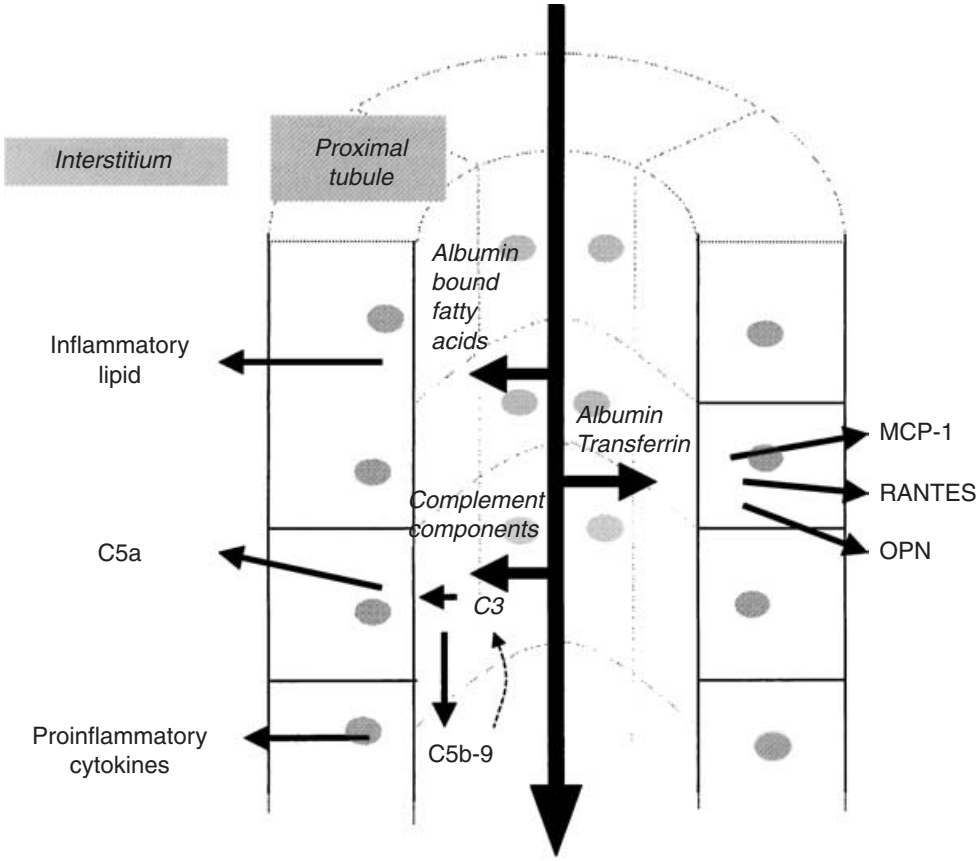


**Fig. 4. Proteinuria theory.** Schemata of mechanisms by which proteinuria promotes Mϕ recruitment to the interstitium.

of ACE inhibition may be through reduction of serum aldosterone levels [111]. Clinical studies are assessing if combined ACE and aldosterone inhibition confers additional benefit. This has already been demonstrated in cardiac disease [111].

## What mechanisms link Mϕ recruitment and tubulointerstitial disease progression?

Three major mechanisms of progressive tubulointerstitial injury have been proposed. Their promotion of Mϕ recruitment and activation may provide an important link between the trigger for injury and the establishment of progressive damage.

*Proteinuria.* All low-molecular-weight (MW) proteins (<40 kD) and a fraction of intermediate MW proteins (40–100 kD), such as albumin, pass across the glomerular basement membrane into the urinary space (reviewed in [113]). Under normal physiologic conditions, very little protein is detected in the urine due to highly efficient reabsorption by proximal tubular epithelial cells. However, in glomerular disease, loss of basement membrane integrity increases leakage of intermediate MW proteins, and high MW proteins also begin to cross the basement membrane. Thus, tubular epithelial cells are both exposed to higher concentrations and endocytoze a different repertoire of proteins. This protein overload exceeds the normal reabsorptive capacity of these cells and affects their functional integrity with direct biological consequences, including the recruitment and activation of mononuclear cells by several pathways (Fig. 4): (*1*) in vitro proximal TECs express Mϕ-directed chemokines in response to high concentrations of proteins of intermediate-molecular-weight, including albumin and transferrin [114, 115]. Further, in human disease, urinary levels of chemoattractants have been correlated with the degree of proteinuria [37]. Interventions that decrease proteinuria in animal models of disease are associated with reduced tubular uptake of protein, chemokine expression, and inflammation (Table 4); (*2*) in response to an increasing protein uptake, there is increased tubular cell production of ammonia, which may directly increase tubular C3 convertase activity and so generate Mϕ chemoattractants and other complement activation products [70]. Urinary levels of complement activation products correlate with the degree of proteinuria in a range of renal diseases [70]; and (*3*) urinary proteins activate TEC production of angiotensinogen and ACE with subsequent increased local production of Ang II [116]. In addition to inflammatory effects that promote Mϕ recruitment (see

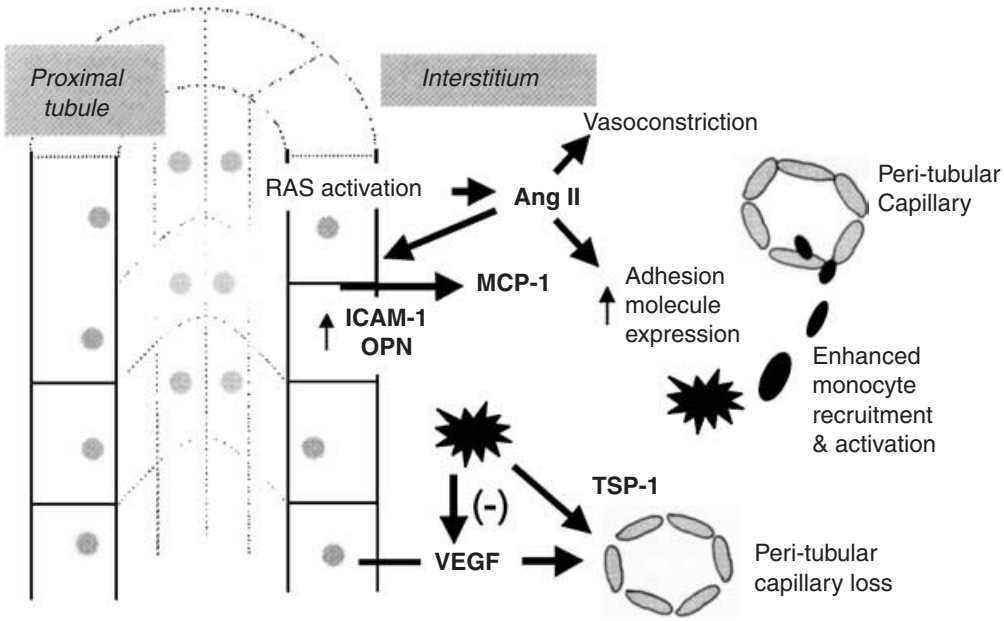


**Fig. 5. Hypoxia theory.** Schemata of mechanisms by which hypoxia promotes Mϕ recruitment to the tubulointerstitium, which, in turn, exacerbates tissue hypoxia.

above), Ang II is best known as a potent vasoconstrictor. This may lead to glomerular hypertension in previously unaffected nephrons. These glomeruli would ultrafiltrate protein and potentiate the chemotactic potential of TEC [117].

The demonstration of causation of Mϕ recruitment and tubulointerstitial disease by proteinuria requires study in isolation from other biological effects: this is currently not possible in humans. The animal model that approaches this requirement is the protein-overload proteinuria model. There is concern, however, that the disease induced is related to factors peculiar to the heterogeneous albumin used (see above), although experiments that characterized the model found that homologous protein also induced disease [13].

It is also unclear why patients with minimal change nephropathy do not develop tubulointerstitial disease. This may relate to the selectivity of proteinuria in this disease, as high MW proteins, such as complement, are not leaked. Also, the fatty load of the albumin in MCN is relatively small [101]. These observations may indicate the differential nephropathic effect of different proteins (reviewed in [113]).

*Chronic hypoxia.* In the development of interstitial fibrosis and tubular atrophy, reduced capillary density may promote tissue hypoxia through increasing the effective distance of oxygen transport. The development of interstitial scarring may further exacerbate hypoxia by impairing the diffusion gradient of oxygen. With the generation of ROS by resident renal cells in response to ischemia, proximal TEC and interstitial fibroblasts respond by promoting ECM deposition with subsequent scar formation [118]. Hypoxia can also directly induce tubular cell apoptosis. Futrakul et al have demonstrated an inverse correlation between peritubular capillary blood flow and tubulointerstitial disease [119].

Our understanding of the processes that reduce capillary density and blood flow is incomplete (reviewed in [120]). Putative mechanisms include (Fig. 5): (*1*) vasoconstriction promoted directly by Ang II and endothelin I, or indirectly through loss of vasodilator tone through down regulation of nitric oxide; (*2*) mechanical obstruction to flow by primary glomerular diseases and obliteration of the glomerular capillary bed; and (*3*) changes in local expression of angiogenic growth factors, such as vascular endothelial growth factor (VEGF), during the progression of damage.

Activation of RAS and increased adhesion molecule expression by EC and TEC in response to hypoxia may directly promote Mϕ recruitment [120]; hypoxia may then augment the activation of Mϕ at tissue sites [121]. Recruited Mϕ may themselves promote a hypoxic environment; Mϕ colocalize with tubular cells at sites of VEGF down-regulation, and in vitro, Mϕ-derived cytokines down-regulate tubular VEGF expression [122]. There is de novo interstitial Mϕ and increased TEC expression of thrombospondin-1 (TSP-1) in models of renal disease [122]. This glycoprotein inhibits the proliferation of EC and can induce their apoptosis. In addition to its antiangiogenic effects, it activates locally expressed TGF-β and facilitates the Mϕ uptake of apoptotic cells [123].

For hypoxia to be a primary stimulus for the initiation and progression of renal disease, evidence is required that tubulointerstitial ischaemia precedes the

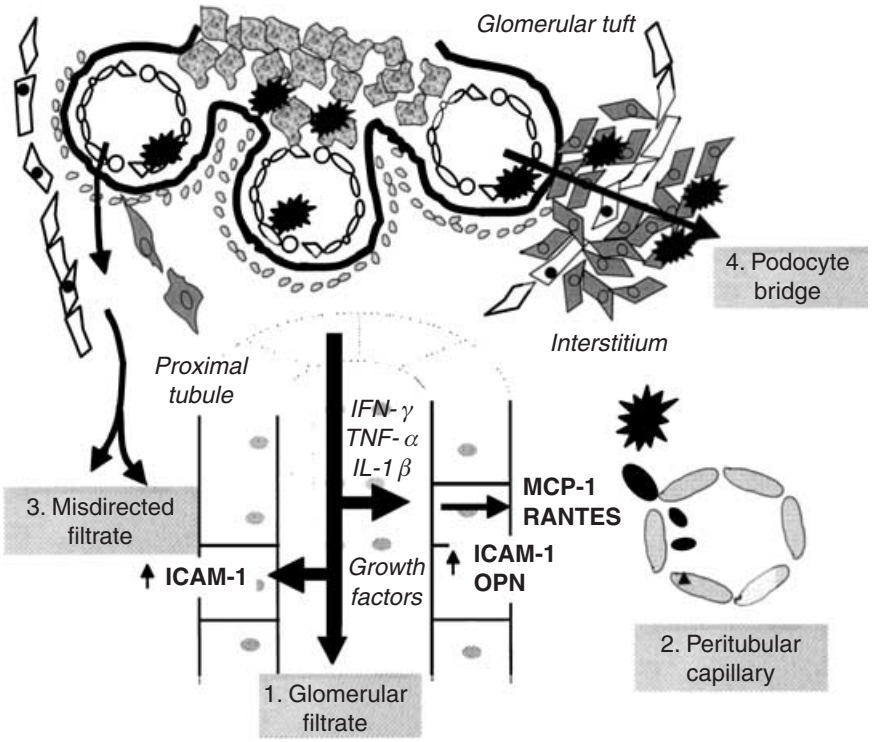


**Fig. 6. Glomerular cytokine theory**. Schemata of the 4 mechanisms by which glomerular-derived cytokines reach the tubulointerstitium, where they may then promote Mϕ recruitment (see text).

development of inflammation and injury [120]. Also, Futrakul et al found reduced interstitial perfusion in patients with progressive forms of nephrotic syndrome prior to the development of TI disease, whereas perfusion was maintained in minimal change nephropathy [119]. In certain renal diseases interstitial ischemia may localize initially to nephrons selected through involvement of their glomeruli in focal disease. Mϕ recruited to these nephrons will then exacerbate local hypoxia and involve the tubules of bystander nephrons, the glomeruli of which may then be exposed to compensatory hemodynamic changes. Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) may work in part through improving interstitial perfusion by antagonizing the vasoactive effects of Ang II. Recent in vivo experiments support this theory [124].

*Glomerular-derived cytokines.* These are synthesized by resident glomerular cells or infiltrating leukocytes (reviewed in [125]), and disperse to tubulointerstitial sites by several routes (Fig. 6): (*1*) via glomerular filtrate to activate tubular epithelial cells; (*2*) through the renal microvasculature to activate endothelial and subendothelial cells; (*3*) through direct communication between the glomerular tuft and Bowman's capsule and periglomerular tubulointerstitium; (*4*) through glomerular tuft lesions that breach the parietal epithelium to lie outside Bowman's capsule. Glomerular filtrate is then misdirected, passing outside Bowman's space to surround the glomerulus and extend along the outer aspect of the proximal tubule to subepithelial peritubular spaces [126–128].

TNF-α, IL-1β, and IFN-γ promote glomerular inflammation and injury and associated tubulointerstitial disease in experimental models of proliferative renal disease (reviewed in [129]). In human proliferative glomerulonephritis, infiltrating and/or resident cells produce these cytokines [129]. There are little data on their expression in situ in chronic glomerular disease, although there is a significant increase in urinary levels in both membranous and IgA nephropathy [130, 131].

The evidence for proinflammatory cytokines and other inflammatory products of glomerular injury in the development of secondary TI disease includes: (*1*) IL-1β increases tubular osteopontin expression, and treatment with an IL-1 receptor antagonist in experimental models of glomerulonephritis ameliorated glomerular injury and reduced tubulointerstitial ICAM-1 expression and leukocyte infiltration [132]; (*2*) TGF-β and Ang II, which are both produced at glomerular sites, increase tubular osteopontin and TNF-α expression, respectively [104, 133]; (*3*) glomerular-derived chemokines may be taken up at the luminal surface by tubular epithelial cells and processed and presented on their basal surface, and/or, in the case of MCP-1/CCL2, increase tubular adhesion molecule and cytokine expression [134]; (*4*) in response to nephron loss, there is increased glomerular expression of proinflammatory molecules, including MCP-1/CCL2, TGF-β, and

adhesion molecules in response to mechanical forces such as shear stress. Soluble mediators produced in this setting may then have downstream effects [3].

## Interstitial macrophage phenotype and function

As interrupting Mϕ recruitment in vivo ameliorates injury in animal models of renal disease, we can infer a proinflammatory Mϕ phenotype in these settings. However, Mϕ also play an important role in tissue repair and remodeling. In this context, local factors are important in determining the phenotype adopted by recruited Mϕ. For example, transgenic mice whose islet cells expressed MCP-1/CCL2 developed a Mϕ insulitis. However, this infiltrate did not promote islet cell destruction, and diabetes mellitus did not develop [135]. Thus, one or more factors necessary for the adoption of a proinflammatory Mϕ phenotype able to promote tissue injury were absent in this model.

These observations are supported by in vitro studies. Treatment with proinflammatory cytokines, including TNF-α and IFN-γ, result in Mϕ adopting a 'classic' activated phenotype. These cells are functionally cytotoxic and express NO, ROS, and proinflammatory cytokines and chemokines (reviewed in [136]). There is also increased expression of HLA class II and CD86. Chemotactic factors involved in Mϕ recruitment, including MCP-1/CCL2, RANTES/CCL5, OPN, and Ang II also increase Mϕ cytotoxicity and proinflammatory cytokine expression [28, 82, 105, 137, 138]. Hypoxia may further augment this response [121].

Treatment with anti-inflammatory cytokines, including IL-4, IL-10, and IL-13, results in 'alternatively' activated Mϕ [136]. This phenotype is associated with the preferential expression of anti-inflammatory cytokines, including IL-10 and IL-1 receptor antagonist (IL-1ra), and the cell surface molecules Mannose receptor and Scavenger receptor. These cells scavenge cellular debris, and promote angiogenesis and tissue remodeling and repair. Recently, a 'type II activated' phenotype has also been described [139]. This occurs on exposure of Mϕ to classic proinflammatory cytokines in the presence of immune complexes; ligation of FCγR prevents the adoption of a 'classic' phenotype, but results in one similar to that observed in the 'alternatively activated' Mϕ.

Other phenotypic markers have been characterized primarily from work using mice [140]. These include FcγR I, II, and III, whose expression is increased when Mϕ are classically activated, and have been used in the study of Mϕ phenotype in human liver disease [141]. The LPS receptor is also highly expressed on classically activated Mϕ in vitro and in vivo [140]. However, analysis of antigenic markers of phenotype by IHC in human renal disease is of limited use because most are not restricted to a specific Mϕ phenotype, and are also expressed by other immune and nonimmune cells.

There is also variation in cell surface antigen expression depending on the tissue site of Mϕ [140]. For example, interstitial, but not glomerular, Mϕ express peroxisome proliferator-activated receptor-γ (PPARγ) in diabetic nephropathy, and CCR5 in glomerulonephritides [42, 142].

Important information on Mϕ phenotype has come from ex vivo analyses. Rees et al have used functional assays on isolated glomerular Mϕ to confirm that the 'classically' activated phenotype predominates in some animal models of glomerular disease [143]. It is more difficult to directly characterize the phenotype of interstitial Mϕ. An increase in expression of proinflammatory over anti-inflammatory cytokines in the tubulointerstitium has not been demonstrated to date, but because the presence of Mϕ at interstitial sites is associated with cell injury, a 'classic' activated phenotype is believed to predominate.

However, there is recent evidence for functional heterogeneity of glomerular and interstitial Mϕ in vivo [136]. The phenotypic profile of Mϕ isolated from the glomeruli in models of proliferative glomerulonephritis depends on the stage of the disease when isolated. Within the interstitium of nonglomerular disease, such heterogeneity may also be important in determining disease outcome. The migration of Mϕ through matrix is facilitated by the fixation of urokinase-type plasminogen activator (uPA) to the cell surface by its receptor uPAR (or CD87); uPA promotes local degradation of matrix proteins by the generation of plasmin. Also, Mϕ-expressed uPAR, through interactions with scavenger receptors, is involved in the scavenging and degradation of profibrotic cytokines and matrix proteins. Using an obstructive uropathy model, Zhang et al have demonstrated that interstitial Mϕ may adopt a disease regulatory phenotype [144]. They showed that uPAR knockout animals had reduced interstitial inflammation but increased fibrosis. Although these data should be interpreted with care, because TECs also expressed uPAR, they may indicate an important antifibrotic and reparative role for a proportion of interstitial Mϕ in TI disease.

Determining the effects of a specific Mϕ-derived gene product on TI disease progression is now possible using the methodology of bone marrow transplantation with genetically engineered stem cells. Nishida et al used this technology to study the role of the Mϕ expression of the Ang II receptor ATR1 in the murine model of obstructive nephropathy [145]. Although its absence on bone marrow–derived cells did not alter the early stages of disease progression, it was associated with reduced interstitial Mϕ infiltration at latter stages. Paradoxically, this was associated with increased scar formation. As ATR1-deficient Mϕ have reduced phagocytic activity, these results imply that Ang II may promote a scavenging

phenotype of a significant proportion of interstitial Mϕ in the latter stages of disease, which is important in the clearance of matrix and apoptotic cells.

Other noncytokine-associated receptors may also have a role in directing Mϕ phenotype. The PPARγ is a member of the nuclear receptors of ligand-activated transcription factors. It is highly expressed in adipose tissues, where it is involved in lipid metabolism and homeostasis. It is also expressed on Mϕ, and receptor ligation downregulates the response of the cell to classic proinflammatory cytokines and promotes the adoption of a scavenger phenotype [146]. The thiazolidinedione group of drugs used in the management of diabetes mellitus in humans is PPARγ agonists. However, when used in an animal model of glomerulonephritis, they had a proinflammatory effect [147].

## Therapeutic strategies targeting interstitial inflammation in secondary TI disease

Results from clinical trials have identified that disease progression can be significantly slowed with aggressive blood pressure control and RAS blockade. A significant proportion of patients, however, still progress to end-stage renal disease despite this approach. These current therapeutic strategies work primarily by counteracting the adverse glomerular haemodynamic changes that occur in disease progression. New approaches may target the development of interstitial inflammation; some of these therapies will directly target Mϕ. The majority of studies of novel biological agents to date have focused on glomerular disease. A benefit in secondary tubulointerstitial disease can be inferred from some of these. We have focused on therapies that directly relate to the areas discussed in this review.

### Chemokine receptor antagonists

There has been a massive biotechnology investment in the development of chemokine receptor antagonists (reviewed in [148]). Their use in animal models of progressive renal disease has yielded encouraging results [64, 68, 69]. However, major challenges have been encountered in their development for human disease; they are closely related to other G protein–coupled receptors, leading to cross reactivity in biological effects. Also, species differences in the expression of chemokines and their receptors make interpretation of results from experiments with animal models difficult. In addition, conformational differences in receptors may mean that antagonists that function well in vitro may not be efficacious in vivo.

Animal models studying the role of chemokine receptors in glomerulonephritides have highlighted a further layer of complexity. Models of anti-GBM disease in mice deficient of the chemokine receptor CCR1 or CCR2 were demonstrated to exacerbate disease [149, 150]. Also, in an immune-complex model of glomerulonephritis, CCR5 blockade aggravated glomerular injury even though leukocyte infiltration was reduced [151]. In this case, partial agonistic effects of the molecule promoted a proinflammatory phenotype of recruited Mϕ. Further, resident renal cells have been demonstrated to express chemokine receptors, making such studies difficult to interpret. Their function on nonimmune cells is not yet defined, but if they have an important homeostatic role, their targeting could promote injury in some settings.

### Complement blockade

These may have a significant therapeutic impact on a wide range of human diseases, including the management of both acute immune-mediated glomerular injury and chronic progressive proteinuric disease (reviewed in [70]). Complement receptor 1–related gene/protein y (Crry) is a naturally occurring cell-bound peptide that inhibits C3/C5 convertase activity. Recombinant technology has led to a soluble incarnation (Crry-Ig), which, on prolonged use in a murine model of lupus nephritis, ameliorates glomerular and interstitial disease [152]. The human corollary of Crry-Ig is soluble complement receptor 1 (sCR1). It is well tolerated by humans, but has not been studied in human renal disease [70].

Complement-specific blocking antibodies are also at an advanced stage of development. Anti-C5 monoclonal antibodies prevent the formation of C5a and C5b-9, and prolonged use in a murine model of lupus disease with nephritis was beneficial [153]. Humanized anti-C5 monoclonal antibodies have been developed and are at an early stage of evaluation in the management of lupus in humans and in membranous nephropathy [70]. The rationale for their use in nonimmune disease has been explored above.

### Optimal RAS blockade

Numerous animal models (see Tables 4 and 5) and clinical trials have demonstrated the beneficial effects of RAS blockade. The more effective the blockade of RAS, the greater the clinical benefit. Patients on ACEIs who achieve a lower blood pressure, significant proteinuria reduction, and demonstrate an early increase in creatinine (of up to 30%) as a marker of decreased intraglomerular pressure have a better renal prognosis than those who do not [154]. There is now increasing evidence for the concomitant use of ACE inhibitors and ARBs [155]. Long-term trials are needed to define the role of drugs that antagonize the effects of aldosterone [111].

### Mycophenolate mofetil

Mycophenolic acid (MPA), the bioactive form of the molecule, was primarily developed to inhibit the

proliferation of T cells, and is used in the prophylaxis of allograft rejection. MMF also directly inhibits the proliferation of Mϕ and resident renal cells. Thus, it may have a potential role in other clinical settings [156]. In animal models of nonimmune renal disease, it arrests progression through inhibiting development of secondary TI disease.

MMF was renoprotective in the subtotal nephrectomy model of progressive disease [157]. The treatment significantly reduced interstitial Mϕ and T-cell numbers and scar formation. Through reducing interstitial inflammation MMF also attenuates the adverse glomerular haemodynamics changes that occur after subtotal nephrectomy. When commenced shortly after the induction of injury, MMF did not completely prevent interstitial Mϕ infiltration and scar formation. However, in combination with a RAS blocker, TI inflammation and injury was almost completely prevented, and even when used in established disease, progression was significantly inhibited [158]. MMF was also renoprotective in other models of nonimmune renal disease, including Ang II infusion, NO synthase inhibition, genetic hypertension, adriamycin, and protein overload proteinuria [159–163]. Randomized controlled trials are required to demonstrate beneficial effects of MMF in human nonimmune native progressive renal diseases.

### Gene therapy

The complexity of the kidney poses particular challenges for targeted and efficient gene transfer to a specific cell type (reviewed in [164]). Antisense oligonucleotide (ODN) technology involves the construction of short fragments of nucleic acid whose sequences are complimentary to specific mRNA. When delivered into the cell, it blocks the translation of the targeted mRNA to protein (reviewed in [165]). ODNs are attractive therapies because: (*1*) they do not require a viral vector, which has associated risks; (*2*) they can be administered into a peripheral vein; (*3*) they are accumulated and active in proximal TECs. Okada et al used specific ODNs in a rodent model of proliferative glomerulonephritis to inhibit proximal TEC expression of MCP-1/CCL2 and OPN. This was associated with decreased Mϕ recruitment to the interstitium [52, 166]. ODN technology has also been successfully used in a model of obstructive uropathy, where it reduced proximal TEC ICAM-1 expression and associated interstitial inflammation and scar formation [47]. The effects of a single dose are relatively short lived (several weeks), which may have implications for the management of chronic progressive renal disease [164].

A newly developed kidney-targeted naked plasmid transfer technique has enabled successful gene transfer into interstitial cells with stable and long-term (months) expression. A gene coding the expression of the protein 7ND, which complexes and inactivates MCP-1/CCL2, has successfully attenuated Mϕ recruitment to the interstitium and TI injury in the protein overload model of chronic renal disease [58].

When systemic delivery of a therapeutic protein is required, skeletal muscle–targeting gene therapy is a particularly attractive technique. It is a relatively easy, cheap, and efficient means of gene transfer to facilitate stable and long-term release of the chosen protein. Such a system has been used successfully in an animal model of ichemic-reperfusion injury of the kidney and obstructive uropathy [63].

Once a Mϕ is committed to a particular phenotype, that cell cannot be reprogrammed by its local environment [136]. Manipulating the microenvironment first encountered by an uncommitted Mϕ on entering the kidney, or directing the phenotype of Mϕ before they infiltrate the kidney are interesting therapeutic concepts [129]. Indeed, genetically modified Mϕ, programmed to express the anti-inflammatory cytokine IL-1ra, localized to the kidney, and ameliorated interstitial inflammation and damage in an obstructive nephropathy model [167]. Such techniques have also been successfully used in reducing glomerular injury in models of proliferative glomerulonephritis [168].

## CONCLUSION

We now have substantial knowledge of the role of the Mϕ in promoting secondary TI inflammation and injury. This has led to targeting by novel therapies. However, these approaches in animal models now require careful demonstration of efficacy in human disease. Also, further study is needed to elucidate potential targets that direct the trafficking of Mϕ. Extended analysis of the interactions between Mϕ and resident tissue cells and matrix, and the effect of the phenotype adopted by the infiltrating cell on tissue repair may further direct our approach to the management of established disease.

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